HUMAN HEPATOCYTES AND USES THEREOF

CROSS-REFERENCE

[0001] This application is a continuation application of U.S. application Ser. No. 16/937,777, filed Jul. 24, 2020, which is a continuation application of U.S. application Ser. No. 15/705,587 filed on Sep. 15, 2017, now U.S. Pat. No. 10,765,704, which is a divisional application of U.S. application Ser. No. 14/952,023, filed on Nov. 25, 2015, now U.S. Pat. No. 9,808,490, which claims the benefit of U.S. Provisional Application No. 62/085,185, filed on Nov. 26, 2014, each of which is incorporated herein by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Dec. 31, 2015, is named 44980-704.201_SL.txt and is 31,606 bytes in size.

INCORPORATION BY REFERENCE

[0003] All publications, patents, and patent applications disclosed herein are incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. In the event of a conflict between a term disclosed herein and a term in an incorporated reference, the term herein controls.

BRIEF SUMMARY

[0004] In one of many aspects, disclosed herein is a method of inducing a trophoblast stem (TS) cell to differentiate into an induced hepatocyte in vitro, comprising: contacting the trophoblast stem cell with a conditioned medium (e.g., for sufficient time) to induce differentiation of the trophoblast stem cell into an induced hepatocyte, wherein the condition medium comprises a fibroblast growth factor (FGF), a steroid, and a cytokine. In some embodiments, disclosed herein is a method of inducing a trophoblast stem (TS) cell to differentiate into an induced hepatocyte in vitro, which comprises (a) contacting the trophoblast stem cell in a conditioned medium comprising a fibroblast growth factor (FGF), a steroid, and a cytokine; and (b) incubating the cell for sufficient time to induce differentiation of the trophoblast stem cell into an induced hepatocyte. In some embodiments, the method further comprises contacting the trophoblast stem cell with the FGF prior to addition of the steroid and the cytokine to the conditioned medium. In some embodiments, the trophoblast stem cell is contacted with FGF for at least 2 hours, at least 4 hours, at least 6 hours, 8 hours, at least 12 hours, at least 16 hours, at least 20 hours, or at least 24 hours prior to addition of the steroid and the cytokine to the conditioned medium. In some embodiments, the method further comprises incubating the trophoblast stem cell for at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, or at least 7 days. In some embodiments, the steroid and the cytokine are added simultaneously or sequentially into the conditioned medium.

[0005] In some embodiments, an induced hepatocyte herein is a hepatic progenitor cell. In some embodiments, FGF upregulates miRNA-124a in the TS cell. In some embodiments, elevated level of miRNA-124a initiates definitive endoderm (DE) specification in the TS cell. In some embodiments, the DE specification is associated with biomarkers comprising forkhead box protein A2 (FOXA2), SRY-box 17 (SOX17), Goosecoid (GSC), or Homeodomain protein MIXL1. In some embodiments, the DE specification is associated with elevated expression levels of SOX17, FOXA2, and GSC. In some embodiments, the elevated expression levels are increased protein expression levels. In some embodiments, the DE specification is associated with a decreased expression level of MIXL1. In some embodiments, the decreased expression level is a decreased protein expression levels. In some embodiments, the elevated protein expression levels of SOX17, FOXA2, and GSC and the decreased protein expression level of MIXL1 are relative to the protein expression levels of SOX17, FOXA2, GSC, and MIXL1 in an equivalent TS cell that has not undergone DE specification. In some embodiments, the DE specification is further associated with elevated expression levels of SOX2, NANOG, and OCT4. In some embodiments, elevated expression levels of SOX2, NANOG, and OCT4 are increased level of protein expressions. In some embodiments, elevated expression levels of SOX2, NANOG, and OCT4 are increased level of gene expressions. In some embodiments, the elevated expression levels of SOX2, NANOG, and OCT4 are relative to the expression levels of SOX2, NANOG, and OCT4 in an equivalent TS cell that has not undergone DE specification. In some embodiments, differentiation induced by a method herein comprises one or more of four stages: primitive streak to definitive endoderm (DE) stage, hepatic specified endoderm stage, hepatoblastic stage, and the fetal and adult hepatocyte cell stage. In some embodiments, one or more biomarkers selected from the group consisting of CXCR4, FOXA2, SOX17, HHEX, TTR, ALB, TAT, CYP7A1, BSEP, SERPINA1, G6PC, ABCC2, C/EBPβ, HNF1α, HNF4α, and any combination thereof express in one or more of the four stages. In some embodiments, one or more biomarkers selected from the group consisting of CXCR4, FOXA2, SOX17, HHEX, and any combination thereof, express at the primitive streak to DE stage. In some embodiments, an expression level of CXCR4, FOXA2, SOX17, and/or HHEX increases at the primitive streak to DE stage, relative to that before the primitive streak to DE stage. In some embodiments, the increased expression level is an increased level of gene expression. In some embodiments, the expression level of CXCR4, FOXA2, SOX17 and/or HHEX increases by about 1-fold and about 10,000 fold higher than that before the primitive streak to DE stage. In some embodiments, the expression level of CXCR4, FOXA2, SOX17 and/or HHEX increases by about 10-fold and about 1000-fold higher than that before the primitive streak to DE stage. In some embodiments, one or more biomarkers selected from the group consisting of SOX17, TTR, ALB, TAT, SERPINA1, CYP7A1, and any combination thereof express in the hepatic specified endoderm stage. In some embodiments, an expression level of SOX17, TTR, ALB, TAT, SERPINA1, and/or CYP7A1 increases at the hepatic specified endoderm stage, relative to that before the hepatic specified endoderm stage. In some embodiments, the increased expression level is an increased level of gene expression. In some embodiments, the expression level of SOX17, TTR, ALB, TAT, SERPINA1, and/or CYP7A1 increases by about 1-fold and about 1000-fold